Parameter	EZE/S10mg (n=67)	PBO/S80mg (n=67)	EZE/A10mg (n=65)	PBO/A80mg (n=62)	EZE/P10mg (n=71)	PBO/P40mg (n=67)	EZE/L10mg (n=65)	PBO/L40mg (n=65)
LDL-C*	-45.5	-44.7	-53.4	-53.8	-33.8	-31.1	-34.2	-30.5
HDL-C*	8.6	8.2	9.0***	2.8	8.4	6.1	7.9	4.8
TG**	-26.1	-22.6	-31.1	-30.6	-22.9	-19.2	-18.8	-15.3

*Mean % change **Median % change ***P<0.05 vs high dose statin (A80)

1083-143 **Does Pretreatment With Statins Before Percutaneus Coronary Intervention Reduce Myonecrosis?**

Su Min Chang, Naji Yazbek, John A. Farmer, Nasser Lakkis, Baylor College of Medicine, Houston, TX

Background: Cardiac enzyme elevation peri PCI carries a worse outcome. **Objective:** Determine if pretreatment with statins prior to PCI reduces enzyme elevation periPCI and other cardiac events (CE). **Methods:** 119 consecutive pts (63 on statins prior to PCI , 56 not)underwent PCI were followed for 6 months. We compared the peri PCI cardiac enzyme elevation above 3 times normal and 6 months CE rate (death, peri PCI myocardial infarction (MI), non fatal MI, target vessels revascularization (TVR) and unstable angina requiring hospitalization) . Of not pretreated pts , 72% were on statins at follow up as compared to 98 % of pretreated pts. **Results:** Pretreated Patients had more history of MI or revascularization (63% vs 43%, p=0.015), hyperlipidemia (80% vs 48% p=0.001), hypertension (83% vs 49% p=0.02) .The rest of baseline characteristics were similar including use of glycoprotein IIb/IIIa inhibitors (60% vs 68% p=NS) and type of lesions. Pretreatment with statins had less periprocedure enzyme elevation (2% vs 10 % p= 0.04) and lower CE rate at 6 months (21% vs 41% p=0.015). After adjusting for 15 baseline characteristics, use of statins prior to PCI was associated with a decrease in the risk of enzyme elevation and CE. (OR 0.2, CI 0.06-0.63, p=0.006). Age > 65 years and type b2/C lesions predicted worse outcome. **Conclusion:** Statins therapy prior to PCI may reduce periprocedure cardiac enzyme elevation and subsequent cardiac events. These results need to be confirmed in prospective randomized trials.

	Statins n=63	No Statins n =56
PeriPCI enzyme elevation	1 2%	6 10%
death	4 6%	2 4%
Non periPCI MI	4 6%	3 6%
TVR	1 2%	6 10%
USA	3 5%	7 13%
CE	13 21%	24 41%

1083-144 **Statins Potentiate the Anticoagulant Effects of Low Molecular Weight Heparin**

Sirisha Puppala, Katherine Zamecki, Jennifer Zimmer, Rohit R. Arora, Biren Bhatt, Jesse Houghton, Mir Chowdhury, Charles R. Spillert, New Jersey Medical School, Newark, NJ

Background: Pravastatin sodium (PS) has been shown to have an anticoagulant effect in patients. We have shown that PS has anticoagulant effects which are unrelated to a tissue factor pathway. Whether PS can potentiate the anticoagulant effects of low molecular weight heparin (LMWH, Dalteparin) is discussed.
Methods: One milliliter of citrated whole blood (CWB) was incubated for 10 minutes at 37 degrees with the following: 20 µl water (control); 16 µg/ml PS; 0.25 U/ml LMWH; 16 µg/ml PS and 0.25 U/ml LMWH combined. The clotting time (sec) was determined on a Sonoclot Coagulation Analyzer, a miniviscometer, which is sensitive to early fibrin polymer generation.
Results: The clotting times are as follows: control 355 ± 74; PS 462 ± 75;
LMWH 636 ± 191 and PS + LMWH 810 ± 119. All values were significantly different from each other (p<0.05 or less).
Conclusion: PS prolongs clotting time as does LMWH when compared to the control. In addition, the combination of PS and LMWH prolongs clotting time when compared to either alone. The enhanced anticoagulant effects of this combination of drugs may also occur when used clinically. A rapid clinical blood clotting assay capable of monitoring these apparently beneficial effects would be an asset.

1083-145 **Variability in the ABCA1 Gene but Not HMG-CoA Reductase Predicts Low-Density Lipoprotein Lowering Effects of Statins**

Gualberto Ruano, Chad Messer, Bradley Dain, Richard Judson, Carol Reed, Antonio Gotto, Genaissance Pharmaceuticals, New Haven, CT, Weill Cornell Medical College, New York, NY

Background: ABCA1 (ATP-binding cassette, sub-family A_ABC1_, member 1) is involved in Tangier disease and is known to play a role in cholesterol homeostasis but not in statin treatment. HMGCR (HMGCoA reductase) is the target for statins. We investigated whether variability in these genes may be involved in statin response using the STRENGTH study, (Statin Response Examined by Genetic Haplotype Markers, a pharmacogenetic study of statin efficacy).
Methods: 425 patients with hyperlipidemia were randomly assigned to 8 weeks of treatment with one of three statins: 80mg/day simvastatin (N=148), 80mg/day atorvastatin (N=139) or 40mg/day pravastatin (N=138). We sequenced the ABCA1 and HMGCR genes in 679 hyperlipidemic patients plus 93 other individuals. We constructed haplotype markers from unphased genotypes (Drysdale, et al, PNAS, **97**:19, 2000). Each marker was tested for an association with LDL reduction using ANCOVA models. Permutation tests were used to adjust for the multiple markers considered.
Results: We discovered 179 SNPs including 30 novel, non-synomymous SNPs in ABCA1. We found a significant association between an ABCA1 haplotype marker and LDL reduction by statin treatment (P<0.0001). The association is also seen in the individual statin groups with p-values ranging from 0.04 to 0.003. The marker includes GLU(1192) ASP plus 4 other SNPs. Patients with ≥1 copy of the marker respond with 10% less LDL reduction than those without the marker. There were no differences in baseline lipids or HDL response between individuals based on this marker.
For HMGCR, sequencing in our cohort revealed 51 SNPs. We detected no association between haplotype markers of HMGCR and statin response.
Conclusion: Genes involved in cholesterol and lipid metabolic diseases should be candidates for pharmacogenetic analysis of drug response. Drug targets, although useful for drug screening, may not influence interindividual differences in drug response.

ORAL CONTRIBUTIONS

804 Genetic Determinants of Atherosclerosis

Monday, March 31, 2003, 9:15 a.m.-10:30 a.m.
McCormick Place, Room S103

9:15 a.m.

804-1 **LOX-1 Polymorphism as a Susceptibility Genetic Marker for Atherosclerosis**

Ruggiero Mango, Fabrizio Clementi, Gianmarco Contino, Giovanni B. Forleo, Paola Borgiani, Annalisa Botta, Annamaria Nardone, Gaetano Chiricolo, Massimo Marchei, Alessia Romeo, Sabina Guarino, Clarissa Cola, Maria Rosaria D'Apice, Massimo Federici, Ibrahim Fahdi, Renato Lauro, Francesco Romeo, Giuseppe Novelli, Jawahar L. Mehta, University of Tor Vergata, Rome, Italy, University of Arkansas for Medical Sciences, Little Rock, AR

Background and Objectives: Atherosclerosis is the principal process contributing to the pathogenesis of coronary artery disease (CAD), cerebral infarction, and peripheral vascular disease. A large number of risk factors such as hypertension, hypercholesterolemia, diabetes, obesity, smoking and shear stress leads to endothelial activation and/or dysfunction, which elicit a series of cellular interactions that culminate in atherogenesis. Several biochemical and functional studies suggest that a lectin-like receptor for oxidized low-density-lipoprotein (ox-DL), termed LOX-1, may be involved in atherogenesis. A recent linkage study performed in a mouse model, identified LOX-1 as candidate susceptibility gene for human atherosclerosis. Aim of this study is to investigate the role of the LOX-1 gene in human atherosclerosis susceptibility through association studies in different populations.
Materials and Methods: We screened a group of 164 Italian individuals with angiographic CAD phenotype (CAD; n=88) or without any angiographically demonstrable disease (CAD-free; n=76) and a group of 35 individuals from Arkansas (CAD, n=14; CAD-free, n=21).
Results: We characterized five different SNPs (SNP 1-5) at the LOX-1 locus in these populations. We demonstrated that SNP4 (A to G transition) correlates with CAD with a high degree of specificity in the Italian population (X^2 = 7,37; P, 0.007; 1df) and reproduced this association also in the American group (X^2 =5.11; P, 0.0237; 1df).
Conclusion: On the basis of these data on the prevalence of LOX-1 SNPs in two different angiographically documented CAD populations, we think that LOX-1 may be a potent candidate gene for atherogenesis and endothelial dysfunction in response to ox-LDL.
Work supported by the Italian Ministry of University and Research (MIUR)

9:30 a.m.

804-2 **Genetic Determinants of Nicotine-Induced Angiogenesis**

Edwin Chang, Yan Wang, Hanh M. Bui, Johannes Jacobi, Christopher Heeschen, James J. Jang, John P. Cooke, Stanford University Medical School, Stanford, CA

Background: Nicotine is a potent angiogenic agent which induces tumor angiogenesis and plaque neovascularization. The angiogenic effect of nicotine occurs at concentrations similar to those found in plasma of moderate smokers. Nicotine action is mediated by endothelial nicotinic cholinergic receptors (nAChR). We hypothesize that stimulation of the nAChR activates signal transduction pathways and transcriptional pathways that are distinct from other angiogenic factors. Methods: We identified nicotine-regulated genes by using subtraction hybridization technology to isolate the differentially expressed genes. We also employed high-throughput microarray transcriptional profiling to examine and validate gene expression profiles. Human micro vascular endothelial cells were treated with vehicle or nicotine (10^{-8}M), and were subjected to PCR-selected subtraction hybridization. Results: Nicotine increased proliferative but decreased apoptotic indices in sub confluent proliferating endothelial cells (HUVEC and HCAECs) with respect to untreated cells. Hexamethonium blocked the anti-apoptotic effect of nicotine. In nicotine

treated cells, a number of differentially expressed transcripts were identified and cloned. Sequence homology search revealed and matched the identified clones to 1) a small subset of genes known to be involved in angiogenesis, e.g. platelet endothelial cell adhesion molecule 1 (PECAM-1), matrix metalloproteinase 2 (MMP2), endothelin converting enzyme 1 (ECE-1), and vascular endothelial growth factor receptor 2 (VEGFR-2); 2) a large subset of known genes with known function, but not involved in angiogenesis to date; and 3) about 5-10% of the clones were novel genes with unknown function, such as a putative G-protein coupled receptor. Thus there are clusters of related gene products, differentially regulated and involved with cholinergic, proliferative and apoptotic action. cDNA microarray analysis (~48,000 elements) validated our findings Conclusion: Nicotine promotes angiogenesis through stimulation of angiogenic mechanisms partly through the cholinergic pathway. Therapeutic modulation of nAChR may be useful in disorders of angiogenesis.

9:45 a.m.

804-3 **Relation Between the $C_{677}T$ Transition in the Methylentetrahydrofolate Reductase Gene, Plasma Homocyst(e)ine and Folate Levels, and Coronary Artery Disease in the GENICA (Genetic and Environmental Factors in Coronary Atherosclerosis) Study**

Gian Paolo Rossi, Maurizio Cesari, Alberto Burlina, Stefania Colonna, Mario Zanchetta, Giuseppe Maiolino, Pietro Maiolino, Achille Cesare Pessina, Clinica Medica 4, Padova, Italy, Cittadella, Italy

Background: Hyperhomocyst(e)inemia has been implicated in atherosclerosis and can be determined by multiple environmental and genetic factors. However, the relationship of the plasma levels of homocyst(e)ine (Ho) and folate (F) with the $C^{677}T$ methylentetrahydrofolate reductase (MTHFR) gene polymorphism and coronary artery disease (CAD) has never been investigated in a large clinical dataset. **Methods:** In 964 consecutive patients (63±10 yrs, 74% men and 26% women) of the "GENICA" study, who underwent coronary angiography for suspected CAD, we measured Ho and F by HPLC and a chemiluminescence method, respectively. All were genotyped for the $C^{677}T$ MTHFR gene polymorphism by fluorescent PCR and melting curve analysis (LightCycler™). **Results:** We found a highly significant ($p<0.0001$) inverse relationship between Ho and F. A multivariate analysis identified serum creatinine, $C^{677}T$ MTHFR genotypes, F, left ventricular ejection fraction, age, and an interaction between $C^{677}T$ MTHFR genotypes and F, as significant predictors of Ho ($R^2=0.16$, $p<0.0001$). When the effect of the T MTHFR allele was examined according to a recessive model, significantly ($p<0.0001$) higher Ho values were seen in TT (14.9±0.6 µmol/l) than in CC+CT (12.0±0.3 µmol/l) patients. At variance no significant difference was seen between patients with (14.0±0.6 µmol/l) and without CAD (13.0±0.4 µmol/l). χ^2 analysis showed that high Ho (>15 µmol/l) were more common than expected in patients with history of previous myocardial infarction ($p=0.003$), peripheral vascular disease ($p<0.001$), vascular surgery ($p<0.001$) and chronic renal failure ($p<0.001$). No associations of the $C^{677}T$ MTHFR polymorphism with such outcomes were seen. **Conclusions**: These results, in patients with angiographically-assessed CAD, support the contention of Ho being determined by multiple factors, including the $C^{677}T$ transition in the MTHFR gene. Furthermore, they indicate that hyperhomocyst(e)inemia, but not the $C^{677}T$ MTHFR alleles, are associated with cardiovascular outcomes albeit not with angiographically assessed CAD.

10:00 a.m.

804-4 **Endothelin-1 Induces Expression of Functional CD40 on Human Vascular Smooth Muscle Cells**

Michael Browatzki, Caroline A. Pfeiffer, Roger Kranzhöfer, University of Heidelberg, Heidelberg, Germany

Background: Chronic inflammation of the vessel wall is a hallmark of atherosclerosis. This inflammatory process is maintained by a variety of cytokines generated in the vessel wall. Recently, activation of vascular cells by cell-cell contact via the CD40/CD154 system has been identified as important pathway of inflammatory stimulation in atherogenesis. Human vascular smooth muscle cells (SMC) as important cellular component of the atherosclerotic plaque can express both cytokines and the CD40/CD154 system. On the other hand, the vasoactve peptide endothelin-1 (ET-1) is supposed to contribute to atherogenesis. This study investigated whether ET-1 stimulates the inflammatory response in SMC via a CD40/CD154 dependent pathway. Methods and Results: ET-1 (10 nM max) like the positive stimulus interferon-gamma (100 U/ml) increased CD40 mRNA and protein expression after 24 hours in human SMC. This ET-1 effect was mediated by the ET-A-receptor subtype since BQ-123, a selective ET-A receptor antagonist, prevented ET-1-induced CD40 upregulation whereas BQ-788, an ET-B-receptor antagonist, did not (10 µM each). ET-1 also activated the proinflammatory transcription factors NF-kappaB and AP-1 in a time dependent manner. The specific proteasome inhibitor PI-1 (50 µM) and a NF-kappaB decoy oligodesoxynucleotide prevented ET-1-induced CD40 expression demonstrating dependence of this ET-1 effect on NF-kappaB activation. To test the functional relevance of the CD40 expression, SMC were preincubated with 10 nM ET-1 for 24 hours and afterwards stimulated with recombinant CD154 (5 ng(ml). Release of interleukin-6 (IL-6) into the culture medium was assessed by ELISA. Cells preincubated with ET-1 secreted a significantly higher amount of IL-6 under CD154 stimulation than control cells (265 +/- 4 vs 147 +/- 8 pg/ml, $p < 0.05$). Conclusion: ET-1 induces an inflammatory response in human SMC via direct cell-cell contact which is mediated by the CD40/CD154 system. This mechanism may contribute to the pathogenesis of atherosclerosis.

10:15 a.m.

804-5 **Decreased Caveolin-1 Expression in Atheroma: Loss of Antiproliferative Control of Vascular Smooth Muscle Cells in Human Atherosclerosis**

Carsten Schwencke, Alexander Schmeisser, Rolf Wachter, Brigitta Weck, Rainer Marquetant, Michael Kasper, Ruth H. Strasser, University of Technology Dresden, Dresden, Germany

Background: Proliferation of vascular smooth muscle cells (VSMC) is involved in the pathogenesis of primary atherosclerosis and restenosis after angioplasty. On the background of the recently proposed antiproliferative activities of caveolin-1 the present study investigated the expression of caveolin-1 in proliferating VSMC in vitro and especially in human atheroma.
Methods and Results: Primary VSMC express high levels of caveolin-1 as shown by immunoblotting. Supplementation of serum or growth factors such as PDGF caused a decrease in caveolin-1 expression in VSMC. Cell-cycle entry was documented by a decrease of the Cdk inhibitor p27kip1 and an increase of the proliferating cell nuclear antigen (PCNA). We further investigated the expression of caveolin-1 in VSMC of human atheroma using immunohistochemistry. In contrast to control vessels, caveolin-1 was markedly decreased in sections derived from human atheroma. The proliferation of VSMC in atheroma was confirmed by an increased PCNA immunostaining.
Conclusion: This newly characterized decreased expression of caveolin-1 both in proliferating smooth muscle cells in vitro and in human atheroma in vivo strongly links the loss of the antiproliferative control by caveolin-1 to the development of atherosclerosis, suggesting a pivotal role of caveolin-1 in the pathogenesis of atherosclerosis.

ORAL CONTRIBUTIONS

811 Pulmonary Hypertension and Pulmonary Embolism: Clinical Insights

Monday, March 31, 2003, 11:00 a.m.-12:15 p.m.
McCormick Place, Room S102

11:00 a.m.

811-1 **Pulmonary Artery Systolic Pressure in Echocardiographically Normal Subjects**

Richard V. Milani, Carl J. Lavie, Yvonne E. Gilliland, Krishnamoorthy Vivekananthan, Mark M. Cassidy, Jose Alberto Bernal, Ali Morshedi, Ochsner Clinic Foundation, New Orleans, LA

Backgrond: Pulmonary hypertension (PHTN) has undergone renewed interest of late with the increasing prevalence of obesity and the reported association of various anorectic agents and its subsequent effect on the pulmonary vasculature. Recent enhancements in echocardiographic instrumentation, has refined the detection of small degrees of tricuspid regurgitation in subjects, and mild "elevations" of pulmonary artery systolic pressure (PASP) are now a common finding, resulting in concern as to whether this represents true pathology. Previous definitions of PHTN suggested that PASP exceeding 30 mmHg were pathologic, however this data was often derived from small numbers of relatively young patients.
Methods: We have analyzed PASP from our echocardiographic database of 35,815 subjects, resulting in 2,472 subjects (mean age 54.9 +/- 16.3 years) which met the echo criteria of normal hearts, defined as: normal left and right ventricular dimension and function; normal left and right atrial dimensions; absence of aortic root dilatation or pericardial disease; absence of valvular stenosis; absence of valvular insufficiency less than moderate.
Results: The mean PASP was 33.1 +/- 7.7 mmHg, and correlations were found with age ($p=0.0001$), male gender ($p=0.0025$), septal wall thickness ($p=0.0001$) and posterior wall thickness ($p=0.0001$). 60% of this population had a PASP > 30mmHg, and 22% of those older than 50 years, and 20% of those with BMI > 30 kg/m2, had a PASP > 40mmHg.
Conclusions: Elevations of PASP is relatively common in echocardiographically normal populations and correlate to age and BMI. Previous definitions of normal PASP should be revised to adjust for age.

11:15 a.m.

811-2 **Inhibition of Phosphodiesterase-5 and Nitric Oxide Similarly Reduce Pulmonary Artery Pressure at High Altitude**

Hans P. Brunner-La Rocca, Patrick Egger, Oliver Senn, Manuel Fischler, Rahel Thalmann, Konrad Bloch, Marco Maggiorini, University Hospital, Zurich, Switzerland, University Hospital, Basel, Switzerland

Background: At high altitude, hypoxia induces pulmonary hypertension which plays an important role in the pathophysiology of high-altitude pulmonary edema. Inhaled nitric oxide (NO) was shown to reduce pulmonary artery pressure (PAP), but it is not applicable in parctice. Therefore, we investigated the effects of the PDE5-inhibitor sildenafil (Sil) on PAP at high altitude in comparison with inhaled NO.
Methods: Doppler-echocardiography was performed in 22 healthy mountaineers (10 W, 12 M, age 29±12y; O_2-saturation 75±3%) 3 hours after they reached an altitude of 4559m. Measurements were repeated after NO (40ppm), Sil (50mg), and Sil plus NO. PAP was estimated from tricuspid regurgitation and pulmonary vascular resistance